

Table of Contents

Contents.....	Page
1 INTRODUCTION.....	1
2 MATERIALS AND METHODS.....	7
2.1 Organisms.....	7
2.1.1 <i>Puccinia triticina</i> isolates.....	7
2.1.2 Plants.....	7
2.2 Developing monopustule isolates.....	7
2.3 Grouping of <i>P. triticina</i> isolates.....	8
2.4 Multiplication and maintenance of <i>P. triticina</i> isolates.....	10
2.5 Growth, inoculation, and incubation of plants.....	10
2.5.1 Plants used for molecular diversity of <i>P. triticina</i> isolates.....	10
2.5.2 Plants used for virulence analysis and gene postulation.....	11
2.5.3 Plants used for mapping <i>Lr38</i> with SSR markers	13
2.5.3.1 Growing parents for crossing.....	13
2.5.3.2 Growing, inoculating, incubating F ₁ , F ₂ , and F ₃ plants.....	14
2.6 Disease assessment.....	15
2.7 DNA extraction.....	16
2.7.1 DNA isolation from <i>P. triticina</i> isolates.....	16
2.7.2 DNA isolation from plants.....	16
2.8 Determination of AFLP genotypes.....	16
2.8.1 PCR amplification.....	16
2.8.1.1 Digestion of template DNA with <i>Tru9I</i> and <i>EcoRI</i> enzymes.....	16
2.8.1.2 Adaptor ligation.....	17
2.8.1.3 Pre-amplification.....	17
2.8.1.4 Selective amplification.....	18
2.8.2 Electrophoresis of AFLPs.....	18
2.8.3 Validity of AFLP technique.....	19
2.9 Virulence analysis and gene postulation.....	19
2.9.1 Virulence analysis.....	19
2.9.2 Gene postulation.....	20

2.10 Mapping of the gene <i>Lr38</i> using SSR markers.....	20
2.10.1 SSR analysis.....	21
2.10.1.1 SSR analysis in Li-COR system.....	21
2.10.1.2 SSR analysis silver staining technique.....	22
2.11 Scoring DNA fragments/bands.....	24
2.11.1 Scoring AFLP fragments.....	24
2.11.2 Scoring SSR bands.....	24
2.12 Statistical analysis.....	24
2.12.1 Molecular diversity of <i>P. triticina</i> isolates.....	24
2.12.1.1 Genetic similarity.....	24
2.12.1.2 Cluster analysis.....	24
2.12.1.3 Principal coordinate analysis.....	25
2.12.1.4 Allele frequency.....	25
2.12.1.5 Grouping of <i>P. triticina</i> isolates.....	25
2.12.1.6 Virulence similarity.....	25
2.12.2 Correlation between genetic and virulence similarity matrices.....	26
2.12.3 Linkage analysis for the SSR markers.....	26
3 RESULTS.....	27
3.1 Molecular diversity of <i>P. triticina</i> isolates.....	27
3.1.1 Validity of AFLP data.....	28
3.1.2 Overall and average country GS.....	29
3.1.3 Grouping of <i>P. triticina</i> isolates.....	30
3.1.4 Cluster analysis.....	31
3.1.5 Principal coordinate analysis (PCoA).....	32
3.1.6 Allele frequency.....	33
3.2 Virulence analysis and gene postulation.....	35
3.2.1 Virulence phenotypes (races) of <i>P. triticina</i> isolates.....	35
3.2.2 Postulated leaf rust resistance genes.....	40
3.3 Relationship between genetic and virulence similarity matrices.....	46
3.4 Mapping of the gene <i>Lr38</i> with SSR markers.....	48
3.4.1 Segregation of resistance to leaf rust.....	49

3.4.2 Identification of SSR markers linked to the gene <i>Lr38</i>	50
3.4.3 Linkage analysis.....	52
4 DISCUSSION.....	53
4.1 Molecular genetic variation of <i>P. triticina</i> isolates.....	53
4.2 Virulence analysis and gene postulation.....	57
4.3 Relationship between genetic and virulence similarity matrices.....	60
4.4 Mapping of the gene <i>Lr38</i> with SSR markers.....	62
5 SUMMARY.....	64
6 REFERENCES.....	67